

Amendment

IN THE SPECIFICATION

Page 1, line 2, please delete "1989" and insert therefor

-- 1990 --;

line 4, after "1986", please insert --/ now abandoned,

but of which U.S. Serial No. 627,707, filed December

14, 1990, is a continuation, and --;

Page 20, line 24, please delete the word "leukemeia", and insert

therefor -- leukemia --;

Page 28, line 13, please delete the word "chomosome" and insert

therefore -- chromosome --;

Page 51, line 3, please delete the abbreviation "Pn" and insert

therefor -- PN --;

Page 73, lines 25-26, please insert after "Nakane et al."

-- ACTA Histochem. Cytochem., --; and

Page 102, line 17, please delete the word "infared" and insert

therefore -- infrared --.

IN THE CLAIMS

Please cancel Claims 1-9, 20, 21, 23, 27-35, 43-44, 49-50, 54-73, 76-101, and 104-126.

10. (Amended) A method [according to Claim 9] of staining targeted chromosomal DNA that can be used to stain a particular chromosome type or portion thereof, or a particular group of chromosome types or portions thereof, whether the

targeted sequences are present at normal copy numbers for diploid or haploid cells or at higher copy numbers, and wherein the staining pattern produced is indicative of the presence or absence of one or more genetic [rearrangement is] rearrangements diagnostic for chronic myelogenous leukemia (CML), the method comprising the steps of:

*Part 1*  
providing a heterogeneous mixture that contains labeled nucleic acid fragments that are substantially complementary to unique sequence regions of a complexity of at least 35 kilobases (kb) in the targeted chromosomal DNA;

disabling the hybridization capacity of repetitive sequences within said heterogeneous mixture;

reacting the heterogeneous mixture with the targeted chromosomal DNA by in situ hybridization; and

rendering visible the hybridized, labeled nucleic acid fragments.

11. (Amended) A method according to Claim 10 wherein [the] said one or more genetic [rearrangement] rearrangements is or are selected from the group consisting of translocations, amplifications and insertions.

12. (Amended) A method according to Claim 11 wherein said labeled nucleic acid [probes] fragments are [homologous] substantially complementary to [nucleic acid sequences in the

vicinity of the translocation breakpoint regions of] unique sequence regions that flank and/or extend partially or fully across breakpoint regions known to be associated with genetic rearrangements identified with CML in chromosomal regions 9q34 and 22q11 of the human genome [associated with CML].

13. (Amended) A method according to Claim 12 [wherein said nucleic acid probes produce] that produces a staining pattern which is distinctively altered when the BCR-ABL fusion characteristic of CML occurs.

14. (Amended) A method according to Claim 12 [wherein signals from said nucleic acid probes when hybridized to said nucleic acid sequences produce] that produces staining patterns as represented in Figure 11, section b-e, inclusively.

16. (Amended) A method according to Claim 15 wherein [the] a portion of [the probe to] said heterogeneous mixture contains nucleic acid fragments that are substantially complementary to unique sequences within the BCR region [is] and are labeled/visualized in one manner and [the] a portion of [the probe to] said heterogeneous mixture contains nucleic acid fragments that are substantially complementary to unique sequences within the ABL region [is] and are labeled/visualized in another manner so that when the BCR-ABL fusion is present, the

proximity of said two labeling/ visualization means become relatively close in an interphase and/or metaphase chromosomal spread.

B3  
17. (Amended) A method ~~according~~ according to Claim [14] 10 wherein said [nucleic probes] unique sequence regions have a complexity of from about 50 kilobases (kb) to about 1 megabase (Mb).

B4  
19. (Amended) A method ~~[according]~~ according to Claim 18 wherein the complexity is from about 200 kb to about 400 kb.

B3  
22. (Amended) Nucleic acid probes, for use in in situ hybridization to detect one or more genetic rearrangements that are diagnostic for chronic myelogenous leukemia (CML), that reliably stain targeted chromosomal [materials] DNA, [wherein said targeted chromosomal materials are in the vicinity of one or more suspected genetic rearrangements] comprising a heterogeneous mixture that contains labeled nucleic acid fragments that are substantially complementary to targeted unique sequence regions on chromosome 9 and/or chromosome 22, which mixture either is substantially free of repetitive nucleic acid sequences or contains sufficient unlabeled repetitive nucleic acid sequences to inhibit substantial binding of the labeled repetitive nucleic acid sequences to repetitive nucleic acid sequences in said

b5  
cont

chromosomes, such that the targeted sequences bind sufficiently more labeled probe during in situ hybridization than nontargeted sequences, so that they can be detected and distinguished from nontargeted sequences whether the targeted sequences are present at copy numbers that are normal for haploid or diploid cells or are present at higher copy numbers.

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b4

24. (Amended) Nucleic acid probes according to Claim [23 wherein said nucleic acid sequences] 22 of sufficient complexity to stain reliably each of two or more target sites on chromosomal [material] DNA [in a genome].

25. (Amended) Nucleic acid probes according to Claim 24 which are substantially free of nucleic acid sequences having hybridization capacity to sites on non-targeted chromosomal [material] DNA.

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b1

36. (Amended) [High complexity nucleic] Nucleic acid probes [according to Claim 35 wherein said], for use in in situ hybridization to detect one or more genetic rearrangements that are diagnostic for chronic myelogeneous leukemia (CML) comprising a heterogeneous mixture that contains labeled nucleic acid fragments that are substantially complementary to targeted unique sequence regions of total complexity of at least 35 kilobases (kb) on chromosome 9 and/or chromosome 22, which mixture either

is substantially free of repetitive nucleic acid sequences or contains sufficient unlabeled repetitive nucleic acid sequences to inhibit substantial binding of the labeled repetitive nucleic acid sequences to repetitive nucleic acid sequences in said chromosomes, such that the targeted sequences bind sufficiently more labeled probe during in situ hybridization than nontargeted sequences, so that they can be detected and distinguished from nontargeted sequences whether the targeted sequences are present at copy numbers that are normal for haploid or diploid cells or are present at higher copy numbers.

37. (Amended) [High complexity nucleic] Nucleic acid probes according to Claim 36 wherein said genetic rearrangements are selected from the group consisting of translocations, insertions and amplifications.

38. (Amended) [High complexity nucleic] Nucleic acid probes according to Claim 37 which produce a staining pattern which is distinctively altered when the BCR-ABL fusion characteristic of CML occurs.

39. (Amended) [High complexity nucleic] Nucleic acid probes according to Claim 38 which have a complexity of from about 50 kb to about 1 megabase.

40. (Amended) [High complexity nucleic] Nucleic acid probes according to Claim 39 wherein the complexity is from about 50 kb to about 750 kb.

41. (Amended) [High complexity nucleic] Nucleic acid probes according to Claim 40 wherein the complexity is from about 200 kb to about 400 kb.

*Handwritten: BCR-ABL*

42. (Amended) A method of detecting one or more genetic rearrangements diagnostic for CML comprising the steps of:

a. hybridizing the probes of Claim [32] 36 to targeted chromosomal DNA [material in the vicinity of a suspected genetic rearrangement];

b. observing and/or measuring the proximity of and/or other characteristics of the signals from said probes; and

c. determining from said observations and/or measurements obtained in step b) whether a genetic rearrangement diagnostic for CML has occurred.

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*Handwritten: BCR-ABL*

45. (Amended) A method according to Claim [44] 42 wherein [the cancer is CML and] the genetic rearrangement produces a BCR-ABL fusion.

46. (Amended) A method according to Claim 42 wherein the chromosomal [material] DNA is either in metaphase or in interphase.

47. (Amended) A method according to Claim 46 wherein the chromosomal [material] DNA is in metaphase.

48. (Amended) A method according to Claim 46 wherein the chromosomal [material] DNA is in interphase.

sub D2  
51. (Amended) A method according to Claim [50] 42 wherein staining patterns produced therefrom are used to distinguish normal and malignant cells for purposes of prognosis and/or determining the effectiveness of therapy.

52. (Amended) A method according to Claim 51 wherein regimens for said therapy [therapeutic regimens] are selected from the group consisting of chemotherapy, radiation, surgery and transplantation.

sub D3  
53. (Amended) A method according to Claim [50] 42 wherein staining patterns produced therefrom are useful in monitoring the status of a patient whose chromosomal material is so tested on a cell to cell basis.

39  
74. (Amended) [The chromosome-specific staining reagent of Claim 73] Nucleic acid probes according to Claim 36 wherein said nucleic acid fragments are labeled with radioactive, enzymatic, immunoreactive, fluorochromes and/or affinity detectable reagents.

75. (Amended) [The chromosome-specific staining reagent of Claim 74] Nucleic acid probes according to Claim 36 wherein said fragments are biotinylated, modified with N-acetoxy-N-2-acetylaminofluorene, modified with fluorescein isothiocyanate, modified with mercury/TNP ligand, sulfonated, [digoxigenenated] digoxigeninated, or contain T-T dimers.

B10  
102. (Amended) Test kits comprising the probes of Claim [32] 22.

103. (Amended) Test kits comprising the probes of Claim [38] 36.

Please add the following new claims:

B11  
127. The method according to Claim 10 wherein the hybridization capacity of the repetitive sequences within the heterogeneous mixture is disabled at the same time as said heterogeneous mixture is reacted with the targeted chromosomal DNA by in situ hybridization.